*Proc. Natl. Acad. Sci. USA*
Vol. 80, pp. 1947–1950, April 1983
Cell Biology

# Expression of cellular *myc* and *mos* genes in undifferentiated B cell lymphomas of Burkitt and non-Burkitt types

(*onc* genes/Burkitt lymphomas/8;14 chromosome translocation/RNA blot analysis)

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*Communicated by Edward V. Evarts, January 10, 1983*

**ABSTRACT** **Burkitt lymphomas contain reciprocal translocations between chromosome 8 and one of the chromosomes containing the immunoglobulin gene loci, prompting speculation that consequent activation of a crucial gene(s) on chromosome 8 might be involved in the generation of these tumors. Recently the human counterparts of the retroviral oncogenes v-*myc* and v-*mos* have been mapped to chromosome 8. We have, therefore, analyzed the level of transcription of the cellular *myc* and *mos* genes in a variety of undifferentiated B cell lymphomas of Burkitt and non-Burkitt type that possess either an 8;14 or an 8;22 translocation. These lines expressed 2- to 5-fold more *myc*-specific RNA than do B cell lines without a translocation. Tumor cell lines of American origin with an 8;14 or 8;22 translocation expressed similar amounts of *myc*-specific RNA. Tumor cell lines of African origin contained slightly higher levels of *myc*-specific RNA than did those of American origin. However, level of expression does not appear to correlate with the presence or absence of Epstein–Barr virus. Therefore, a major increase in the transcription of this gene secondary to translocation is unlikely to be the cause of Burkitt lymphoma. There was no evidence of *mos*-related transcripts in any of the cell lines tested.**

Both Epstein–Barr virus (EBV)-positive and -negative Burkitt lymphoma cells carry a specific reciprocal translocation between chromosome 8 and chromosome 14 (1, 2). Recently variant forms of Burkitt lymphoma have been described in which translocations occur between chromosome 8 and chromosomes 2 or 22 (3, 4). Studies of somatic cell hybrids between rodent cells and human B cells, in addition to *in situ* hybridization analyses, have shown that the human immunoglobulin heavy chain gene locus is located on human chromosome 14, specifically band 14q32 (5, 6). The genes for human λ and κ immunoglobulin light chains have been mapped to chromosomes 22 and 2, respectively (7, 8). Therefore Burkitt lymphoma translocations involve chromosome 8 and a chromosome carrying an immunoglobulin gene. In the Daudi Burkitt lymphoma cell line the break in chromosome 14 has been localized to the variable region of the immunoglobulin heavy chain ($V_H$ region), resulting in the translocation of a portion of the $V_H$ gene region to chromosome 8 (9). Moreover the κ immunoglobulin light chain gene is present on the short arm of chromosome 2 (band p12) in the region where the translocation occurs in Burkitt lymphoma variants (10).

The consistent involvement of chromosome 8 in these tumors has led to the speculation that activation of a cellular gene(s) located on the distal fragment of chromosome 8 is responsible for the development of Burkitt lymphoma (11). Recently, the human counterparts of two retroviral oncogenes (c-*myc* and c-*mos*) have been mapped to chromosome 8 (12, 13), specifically to regions on the long arm of chromosome 8 involved in specific translocations in Burkitt lymphomas and a variety of other tumors (14). Furthermore, it is now known that c-*myc* is translocated near the immunoglobulin locus on chromosome 14 in several Burkitt lymphoma cell lines (R. Dalla-Favera, personal communication). Thus available information is suggestive of involvement of c-*myc* in Burkitt lymphomas.

The Moloney murine sarcoma virus, which contains *mos* sequences, produces fibrosarcomas *in vivo* and transforms fibroblasts *in vitro* (15, 16). c-*mos*-specific RNAs have not been detected in uninfected murine cells thus far analyzed (17). However, when the cellular *mos* sequences of the mouse are linked to a viral long terminal repeat, they are capable of transforming NIH 3T3 cells in DNA transfection assays (18). The human cellular *mos* gene has been found inactive in DNA transfection experiments even when linked to the Moloney murine sarcoma virus long terminal repeat elements (19).

In this report we have analyzed the transcriptional activity of the human c-*myc* and c-*mos* genes in a variety of undifferentiated B cell lymphoma lines of Burkitt and non-Burkitt types, in addition to B cell lines that lack the translocations associated with these tumors. These include EBV-infected cord blood lymphocytes and cell lines obtained from patients with infectious mononucleosis. c-*myc*-specific RNA was expressed in all lines examined, with the lymphoma lines consistently expressing slightly higher (2- to 5-fold) levels than lines without any translocation. There was no evidence of c-*mos* expression in any of the lines tested.

## MATERIALS AND METHODS

**Cell Lines.** Cell lines used in this study were provided by Ian Magrath (Pediatric Oncology Branch, National Cancer Institute). Many of the growth and surface marker characteristics of these cells have been recently described (20–22). Cells were passaged in RPMI 1640 medium with glutamine and penicillin/streptomycin with 20% fetal calf serum (GIBCO).

**Probes and Nick Translation.** The 3.5-kilobase (kb) *Sac* I fragment of c-*myc* (chicken) was used in this study. The 2.75-kb *Eco*RI fragment of hu-*mos* (human) (pHM2A) was the gift of George Vande Woude (Laboratory of Molecular Oncology, National Cancer Institute). The cloning of these genes has been described (19, 23). The probes were nick-translated to specific activities of approximately $2 \times 10^8$ cpm/μg of cloned DNA as described (24).

**RNA Isolation, Fractionation, and Hybridization.** Total cellular RNA was extracted from approximately $2 \times 10^9$ cells in the logarithmic phase of growth by using a modified guanidine hydrochloride extraction technique (25). The poly(A)-containing fraction was twice purified by chromatography on oligo(dT)-cellulose columns (26).

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Abbreviations: EBV, Epstein–Barr virus; EBNA, EBV nuclear antigen; kb, kilobase(s); NaCl/Cit, 0.15 M NaCl/0.015 M sodium citrate.

Five micrograms of poly(A)-selected RNAs was fractionated by electrophoresis on horizontal agarose gels containing 2.2 M formaldehyde (27, 28) or 10 mM methylmercury hydroxide (29). After electrophoresis RNAs were stained in 0.2 M ammonium acetate containing ethidium bromide (5 μg/ml), destained after several rinses in distilled water, and photographed under UV light. RNAs were blotted directly on nitrocellulose filters (Schleicher & Schuell, type HA85) with 20× NaCl/Cit (1× NaCl/Cit is 0.15 M sodium chloride/0.015 M sodium citrate) (30). Filters were baked overnight at 60°C or for 2 hr at 80°C in a vacuum oven. Filter-bound DNA was hybridized overnight at 60°C with the appropriate $^{32}$P-labeled probe in a solution consisting of 50% (vol/vol) formamide, 5× NaCl/Cit, 0.045 M $Na_2HPO_4$, 0.005 M $NaH_2PO_4$, denatured yeast RNA (type III) at 400 μg/ml, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 0.1% sodium dodecyl sulfate.

## RESULTS

**Detection and Quantitation of c-*myc* RNA in B Cell Lines.** Table 1 summarizes the various B cell lines used in this study. The cell lines are divided into five categories and include EBV nuclear antigen (EBNA)-negative American Burkitt and non-Burkitt lymphoma lines with an 8;14 translocation, EBNA-positive American Burkitt lymphoma lines with an 8;22 translocation, EBNA-positive African Burkitt lymphoma lines with an 8;14 translocation, EBNA-positive cord blood lymphocyte lines, and EBNA-positive B cell lines from patients in the acute phase of infectious mononucleosis.

To determine the size and relative quantity of c-*myc* specific RNAs transcribed in the B cell lines, poly(A)-selected RNA was isolated from whole cells, denatured, and separated by electrophoresis through formaldehyde/agarose gels. The gels were stained with ethidium bromide to verify that equal concentrations of intact RNA were used in comparative analyses prior to transfer onto nitrocellulose membranes as described in *Materials and Methods*. The transfer of RNA species onto nitrocellulose was complete for species less than 4.8 kb.

The c-*myc* probe detected a major 2.7-kb transcript in all lines tested (Fig. 1). Occasionally minor bands of various sizes between 5 and 6 kb were detected with this probe, but they were not considered further due to their highly inconsistent presence. The intensity of the 2.7-kb band was slightly increased (2- to 5-fold) in the tumor lines when compared to the translocation-negative cord blood and infectious mononucleosis lines. These results were confirmed in experiments in which a 2- to 5-fold dilution of tumor cell RNA was necessary to bring the intensity of the c-*myc* band to the level of control RNA (Fig. 2). The lymphomas of American origin expressed similar amounts of c-*myc*-specific RNA whether they possessed an 8;14 or an 8;22 translocation (compare the first three lanes with the last two lanes in Fig. 1). The tumor lines of African origin expressed slightly more c-*myc*-related RNA than those of American origin. No comparable amplification of the human c-*myc*-specific DNA sequences was demonstrated (data not shown). The presence of EBV does not appear to directly enhance c-*myc* expression as demonstrated by a comparison of EBNA-positive and -negative American lymphoma lines. However, the absence of EBNA expression in cell lines does not necessarily preclude the presence of EBV DNA sequences other than those encoding the EBNA gene product. c-*myc* expression in the HL60 human promyelocytic leukemia cell line is increased approximately 10-fold over control cell lines, as previously reported (31).

**Lack of c-*mos* RNA Expression in Human B Cell Lines.** To determine the relative expression of c-*mos* RNA in the B cell

Table 1. Derivation and EBNA status of cell lines*

| Cell line | Code | Classification | EBNA | Translocation |
|---|---|---|---|---|
| Landis | JLPC 119 | American Burkitt lymphoma | − | 8;14 |
| JD-PB | JD 38 | Undifferentiated lymphoma | − | 8;14 |
| JD-A | JD 39 | Undifferentiated lymphoma | − | 8;14 |
| BM-1 | PA 682 BM 1 | American Burkitt lymphoma | + | 8;22 |
| BM-2 | PA 682 BM 2 | American Burkitt lymphoma | + | 8;22 |
| PE-1 | PA 682 PE 1 | American Burkitt lymphoma | + | 8;22 |
| PE-2 | PA 682 PE 2 | American Burkitt lymphoma | + | 8;22 |
| Raji | Raji | African Burkitt lymphoma | + | 8;14 |
| Daudi | Daudi | African Burkitt lymphoma | + | 8;14 |
| Namalva | Namalva | African Burkitt lymphoma | + | 8;14 |
| Eckhert | IME 182 | Infectious mononucleosis | + | — |
| Hotz | IMH 182 | Infectious mononucleosis | + | — |
| Bell | IMB 182 | Infectious mononucleosis | + | — |
| CB 23 | CB 23 | Cord blood lymphocytes | + | — |

*I. T. Magrath and J. Whang-Peng, personal communication; and see refs. 20–22.

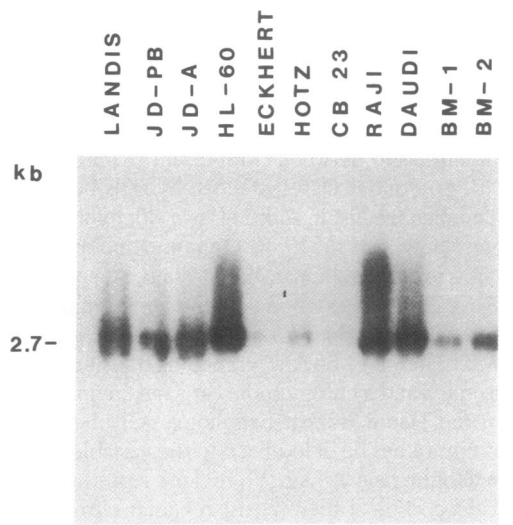


FIG. 1. Hybridization of c-*myc* (chicken) to poly(A)-selected RNAs. Poly(A)-selected RNAs (5 μg) were fractionated by electrophoresis on horizontal agarose gels containing 2.2 M formaldehyde. RNAs were transferred to a nitrocellulose filter and hybridized to a $^{32}$P-labeled cloned nick-translated c-*myc* probe. Landis, JD-PB, and JD-A are EBNA-negative tumor cell lines of American origin containing an 8;14 translocation. Eckhert and Hotz are EBNA-positive translocation-negative B cell lines from patients with infectious mononucleosis. CB 23 is an EBNA-positive translocation-negative cord blood B lymphocyte line. Raji and Daudi are EBNA-positive African Burkitt lymphoma lines that contain an 8;14 translocation. BM-1 and BM-2 are separate lines derived from the same patient and are EBNA-positive American Burkitt lymphoma lines with an 8;22 translocation. HL-60 is a human promyelocytic leukemia cell line and was the gift of R. C. Gallo (National Cancer Institute).

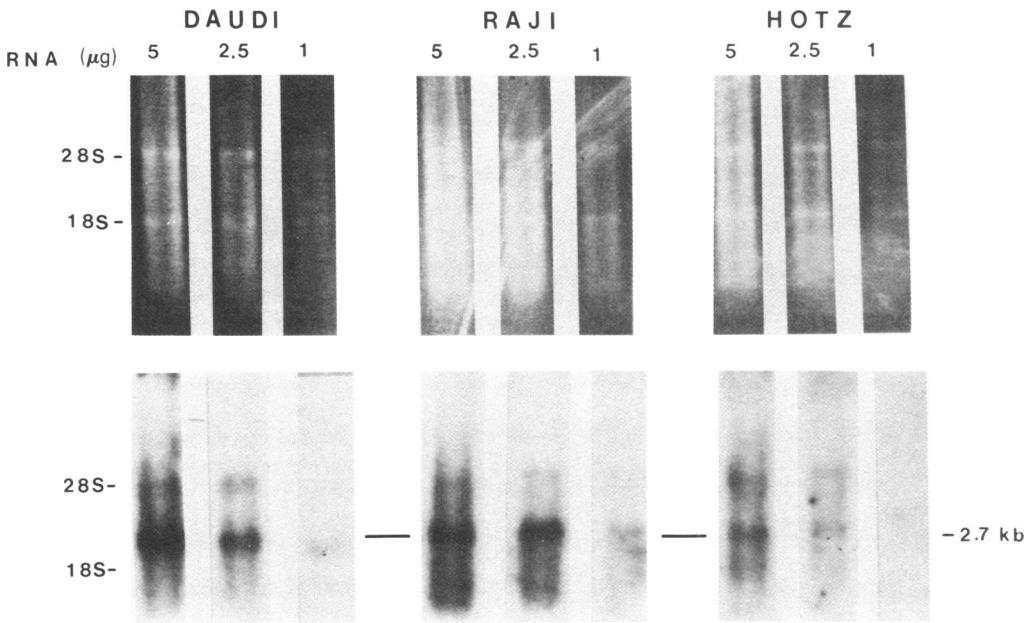


FIG. 2. Hybridization of c-*myc* (chicken) to dilutions of poly(A)-selected RNAs. Various amounts (5, 2.5, or 1 μg) of poly(A)-selected RNAs were fractionated by electrophoresis on horizontal agarose gels containing 2.2 M formaldehyde. RNAs were stained with ethidium bromide and photographed prior to transfer to nitrocellulose filters and hybridization to nick-translated c-*myc* probe. Ethidium bromide stained RNAs pictured above correspond to RNAs directly below, which were hybridized to the c-*myc* probe.

lines, poly(A)-selected RNA isolated from whole cells and pHM2A DNA were denatured and separated by electrophoresis through methylmercury/agarose gels and treated as described above.

No c-*mos*-specific RNA was detected in any of the lines tested (Fig. 3), although the c-*mos* (human) probe was capable of detecting as little as 25 pg of c-*mos*-containing DNA. The presence of intact RNA species was also verified by rehybridization of these filters with c-*myc*, again revealing the presence of the c-*myc*-specific 2.7-kb transcript (data not shown). The hu-*mos* 2.75-kb *Eco*RI DNA fragment was identified in all cell lines (data not shown).

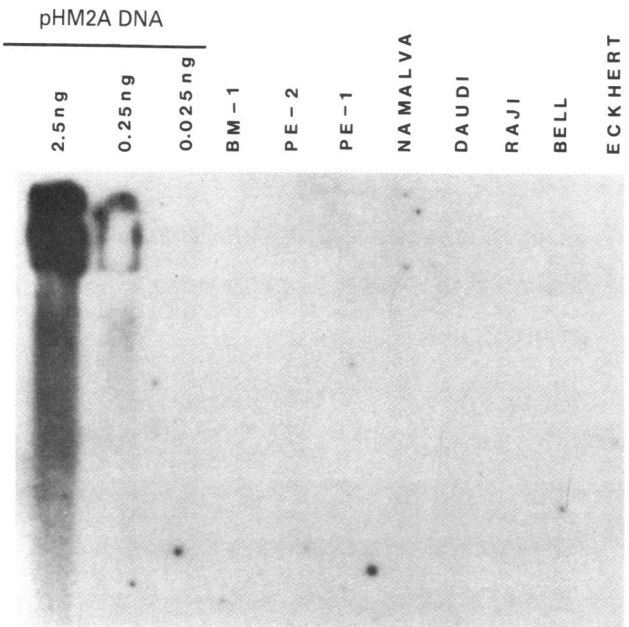


FIG. 3. Lack of hybridization of c-*mos* (human) to poly(A)-selected RNAs. Poly(A)-selected RNAs (5 μg) and 2.5, 0.25, and 0.025 ng of pHM2A DNA were fractionated by electrophoresis on horizontal agarose gels containing 10 mM methylmercury hydroxide. RNAs were transferred to a nitrocellulose filter and hybridized to the $^{32}$P-labeled nick-translated pHM2A c-*mos*-containing probe. BM-1, PE-2, and PE-1 are separate lines derived from the same patient. Cell lines are described in Table 1.

## DISCUSSION

The recent localization of the human c-*myc* and c-*mos* genes to chromosome 8 has led to speculation that activation of one or both of these genes by translocation might be involved in the genesis of Burkitt lymphomas. Activation of c-*myc* by avian leukosis virus is associated with B cell lymphomas of the bursa in chickens (32), a tumor similar in many respects to Burkitt lymphomas of man. Recently Dalla-Favera *et al.* have demonstrated that c-*myc* (human) is translocated very near the immunoglobulin heavy chain locus on chromosome 14 in several Burkitt lymphoma lines studied thus far (R. Dalla-Favera, personal communication). Transfection studies with NIH 3T3 mouse cells, however, do not yield c-*myc* as the transforming element from chicken bursal lymphomas (33). We have similarly failed to find evidence of human c-*myc* sequences in DNAs from NIH 3T3 primary transfectants in which the transformed phenotype has been induced by Burkitt lymphoma DNAs, though the murine c-*myc* sequences can be easily detected (unpublished observation). These data indicate that although activation of c-*myc* may be involved in the oncogenesis of certain B cell lymphomas, other cellular gene(s) derived from either bursal or Burkitt lymphomas have potential oncogenic activity. c-*myc* activation therefore may represent a necessary event in a multistage process leading to tumor formation.

Our data demonstrate a 2- to 5-fold increase in c-*myc* expression by Burkitt lymphoma lines when compared to EBV-"immortalized" B cell lines. No comparable amplification of the human *myc*-specific DNA sequence was demonstrated (data not shown). Tumor cell lines of American origin with 8;14 or 8;22 translocations express similar amounts of c-*myc* RNA. The presence or absence of EBV does not correlate with the level of c-*myc* expression. The slight increase in c-*myc* RNA expression in African lines when compared to those of American origin could possibly be explained by the slightly different stage of B cell differentiation that these tumors represent (34).

In avian leukosis virus-induced bursal lymphomas of the chicken, c-*myc* RNA expression is at least 30-fold increased over control tissues (32). In the HL-60 promyelocytic leukemia cell line c-*myc* RNA expression is increased 10-fold over controls (31), apparently due to an amplification of the c-*myc* gene (35, 36). This amplification is also present in the primary leukemia cells of the patient from which the line was derived, suggesting that increased levels of c-*myc* expression may have been involved in the leukemic transformation of this patient's cells (36).

The small and variable increase in c-*myc* expression among the lymphomas examined in the present study argues against increased transcription of this gene being the cause of Burkitt tumors. In addition it seems that c-*myc* may be translocated to the inactive unrearranged chromosome 14 in these tumors (9), making "up-regulation" by insertion near an active promoter region less likely. The lack of control populations of B cells at the same stage of differentiation as Burkitt tumor cells further challenges the significance of the small differences in c-*myc* expression seen here. Changes in c-*myc* gene environment secondary to translocation might lead to a new or altered promoter region no longer subject to normal regulatory controls. The altered regulation of this gene without markedly increasing its transcription might be associated with tumor formation. Subtle qualitative differences in the c-*myc* transcript in Burkitt tumors is another possibility that cannot be ruled out by our experiments. Alternatively, c-*myc* may not be involved in the generation of Burkitt lymphomas, its translocation in these tumors being a coincidental finding.

There is no evidence of c-*mos* transcription in our cell lines, and it is therefore unlikely that this gene plays a role in the generation of Burkitt lymphomas.

We thank Eric Westin for his critical review of this manuscript and Larry Linder for his excellent technical assistance. We thank Frances Williams for assistance in preparation of the manuscript.

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